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BY

F. D. FROMME AND H. E. THOMAS

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BLACK ROOTROT OF THE APPLE¹

By F. D. FROMME, *Plant Pathologist*, and H. E. THOMAS,² *formerly Assistant Plant Pathologist, Virginia Agricultural Experiment Station*

INTRODUCTION

An unusually destructive rootrot of the apple (*Malus sylvestris*) is prevalent in the chief orchard sections of Virginia. The disease has been known to the growers for a number of years, but it is only quite recently that considerable interest has been manifested in it and a technical study undertaken at the Virginia Experiment Station.

The first published record of the disease, by Reed and Crabill (10) in 1913, contains a brief mention of a rootrot, with the suggestion that the cause may be one or more mushrooms. Subsequent preliminary notes by the same authors (11) and by Crabill (1) have outlined the general symptoms and indicated the importance of the problem. Fulton and Cromwell (5), in an abstract, briefly described a "black rootrot" of apple found in Pennsylvania and North Carolina which appears very similar to and is probably identical with the disease under discussion. In a preliminary note the writers (3, 4) have stated that the rootrot of apple in Virginia is probably due to one or more species of *Xylaria* and have designated it "*Xylaria* rootrot." As a descriptive common name "black rootrot" is preferable and will be used in the belief that the assumed identity of the Virginia and North Carolina rootrot diseases will be confirmed.

The disease is most prevalent in the middle and north "Valley," "Piedmont," and "Appalachian" sections of Virginia, and is probably present to some extent throughout the State. Our information is based on conditions as found in Alleghany, Rockbridge, Albemarle, Nelson, Augusta, Rockingham, Botetourt, Montgomery, and Frederick Counties. Losses of trees ranging between 5 and 25 per cent of the entire orchard have been observed. These losses, together with the high death rate of replants, which will invariably exceed that of the original trees in a stated period of years, make the disease a most formidable one. Financial losses from rootrot are, of course, very considerable.

SYMPTOMS OF ROOTROT

Many of the superficial symptoms of the disease may equally well characterize injuries due to other agencies such as winter injury, malnutrition, attacks of mice or rabbits, tree borers, woolly aphis, crown-gall, and collar-blight; but the combination of all symptoms, particu-

¹ Paper No. 47 from the Laboratories of Plant Pathology and Bacteriology, Virginia Agricultural Experiment Station.

² Now Assistant Plant Pathologist, Porto Rico Agricultural Experiment Station.

larly those of the root system, affords a reliable basis for diagnosis. The first indications of an attack of rootrot are seen in the foliage. The leaves are paler in color than the normal green and have a yellow cast, an appearance of unthriftness, and are smaller than normal. The thinness of the foliage later attracts attention (Pl. 15, A, B). This appearance is produced by a failure of many of the lateral buds to develop and by a check in length of the terminal growths. In later stages one or more of the main branches may die, while others may appear normal, but more commonly the general death of the tree results.

Trees of bearing age in late stages of rootrot have a tendency to heavy bearing, the fruit being small and poor in quality. One of the most reliable signs is an inclination of the trunk, the lean being directed away from the most seriously affected roots. Affected trees appear to die rather suddenly, but in most cases symptoms of rootrot have been present for a year or more; trees are rarely, if ever, killed within a single season from the time of infection. This is shown by a marked check in the terminal growth of branches which usually extends back over a period of two or more years and indicates the cumulative effect of a comparatively slow-acting parasite. Measurements of the length of terminal growths of two contiguous 6-year-old York Imperial apple trees, one healthy and the other affected with rootrot, gave the averages shown in Table I. The affected tree shows a marked check in growth beginning in 1915. Plate 15, E, shows representative terminal growths of these trees; the check in growth, the suppression of lateral buds, and the decrease in number and size of the leaves are apparent.

TABLE I.—Average terminal growth of healthy and of diseased York Imperial apple trees

Year.	Healthy tree.	Rootrot tree.
	<i>Inches.</i>	<i>Inches.</i>
1913.....	11.25	13.00
1914.....	5.25	7.83
1915.....	8.90	.75
1916.....	10.00	1.16

Death from rootrot may result at any stage in seasonal development, but the majority of trees probably succumb during the fall or winter. When death takes place during the growing season, the leaves brown and shrivel and hang on for sometime. Trees of any age may be subject to attack. The greater percentage dies between the ages of 12 and 20 years; a few may die as early as five years or younger. Trees on newly cleared land from which the stumps of forest trees have been removed shortly before planting usually die more rapidly and in greater numbers than those planted on cultivated land. A high percentage of the replants set where trees affected by rootrot have been removed die from the same disease (Pl. 17, B). Replant death commonly begins at three years of

age, and the percentage of death at seven years may equal or exceed that of the original trees at 15 years. All varieties of the apple probably suffer equally from the disease. We have found it equally prevalent on York Imperial, Winesap, Ben Davis, Black Twig, Grimes Golden, Albemarle Pippin (Yellow Newtown), and other varieties under equal conditions of exposure.

The appearance of roots of affected trees is quite characteristic. Young trees in advanced stages of infection may readily be uprooted by hand. In uprooting, the larger roots break off near the trunk, and often only a few small superficial roots support the tree. The bark, when not too far disintegrated, is covered with a thin black encrustation which is easily peeled off. The wood is brown and is marked with dark-brown zonations. On recently killed roots the wood is firm, and the zonations may penetrate it to some depth. The margin between sound and diseased bark and wood is sharply marked by a change in color, and thin brown strands may extend into the sound tissue for some distance. Sunken, discolored areas are usually found on the surface of the sound root beyond the main lesion (Pl. 17, C). They have the appearance of surface infections and may be superficial, or may penetrate to some depth. Usually but little evident mycelium is present on the surface of roots or within them. In advanced stages of decay roots are punky and brittle and vary in appearance; various fungi may be present and acting on them. Rhizomorphoid strands have been found associated with this rootrot in a few cases only, and then on roots in advanced stages of decay. The crown of the tree almost invariably shows the discoloration of the wood and bark found on the roots. The discoloration may extend up the trunk a foot or more above the surface of the ground and is often restricted to one side. Trees showing recognizable foliar symptoms of the disease are found to have the greater part of their root system affected. The initial infection of the root system may take place at any point, and the infection apparently progresses with equal rapidity toward and away from the crown. Infection may be communicated laterally to healthy roots in contact with diseased roots through uninjured rootlets or through wounds in large roots. Infected roots die back to the crown establishing the infection there, and the sound roots become infected subsequently through their attachment with the crown. Death of the tree probably results within a comparatively short time after the infection becomes established in the crown. Figure 1 shows the condition of the root system of a tree which showed pronounced rootrot symptoms.

FUNGI ASSOCIATED WITH ROOTROT

The absence of fruiting stages of fungi on affected roots has been noteworthy. The early removal of diseased trees as practiced in most orchards partly explains this. Perithecial stromata of *Xylaria polymorpha* (Pers.) Grev. have been found on the roots of several affected apple trees in two orchards at Barber (Pl. 15, C; 17, D), and numerous

stromata of *X. hypoxylon* (L.) Grev. appeared at the base of a dead apple tree at Blacksburg (Pl. 17, A). These had produced but few spores at the time of collection (January), but sporulated abundantly after being placed in moist chamber for a month (Pl. 17, F). Carpophores of *Clitocybe monadelphæ* (Morgan) Sacc. were found at the base of several trees in one orchard at Middletown, but the relation of this fungus to the rootrot problem has not been determined. Affected roots brought to the laboratory and placed in moist chamber have developed conidial stromata of *X. hypoxylon* in a few cases (Pl. 15, D).

Isolation studies from roots in various stages of attack have been carried on during the past two growing seasons (1915 and 1916) in con-

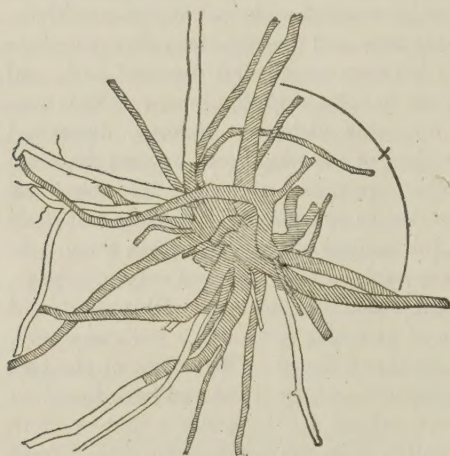


Fig. 1.—Root system of a 7-year-old apple tree affected with rootrot. The infected parts are shaded. Infection was progressing outward on the sound roots. The initial infection probably took place on the side marked X.* Drawn from a photograph.

tinuation of previous studies by Reed and Crabill. Crabill (1) reported the isolation of *Trichoderma koningi* Oud. in culture 72 times in 116 trials, using apple roots from seven sources, and concluded that this fungus is probably the cause of rootrot. No experimental proof of its pathogenicity was given. Jensen (9), Werkenthin (13), and Waksman (12) have found *T. koningi* commonly present in various types of soil, and the writers have found it common on decaying wood. Although we have also frequently isolated this fungus from diseased

apple roots, we are convinced from inoculation studies that it exists in them as a saprophyte in tissue killed by other agencies.

In our isolations we have plated fragments of diseased roots on agar media. In the early part of the work the tissue fragments were taken from various parts of roots and a number of species of fungi were obtained, with *Trichoderma koningi* predominating. Later, the fragments were taken only from the margins of the lesions. A considerable number of these have yielded cultures of a species of *Xylaria*,¹ often uncontaminated with *T. koningi*, the most common accompaniment, and occasional species of *Fusarium*, unidentified fungi, and bacteria. *Physalospora cydoniæ* Arnaud (*Sphaeropsis malorum* Peck) was obtained from the

¹ At first assigned to the genus *Stilbella*. A culture bearing conidial stromata was submitted to Dr. C. L. Shear, of the Bureau of Plant Industry, who stated that the fungus was probably *Xylaria hypoxylon*.

roots of one tree and from the margin of a trunk lesion extending about 1 foot above the surface of the ground on another. *X. polymorpha* was also obtained from both these sources. An unidentified fungus, probably a basidiomycete, which invests the roots with a conspicuous mat of white mycelium has been cultured from several sources. It has proved slightly, if at all, pathogenic on excised living apple roots in a moist chamber, but its appearance in the field suggests that it may be parasitic in some cases, probably on unthrifty trees.

Cultures of species of *Xylaria* were obtained during 1915 and 1916 from orchards located at 11 places in Virginia. Three species are represented.

Xylaria hypoxylon has been obtained from nine places: Winchester, Middletown, Pleasant Valley, Staunton, Fishersville, Cloverdale, Blacksburg, Buena Vista, and Greenwood. The isolations from the last two places are tentatively assigned to this species, but differ from the typical *X. hypoxylon* in certain constant cultural features; whether these are of specific or varietal character remains to be determined.

Xylaria polymorpha has been obtained from two orchards at Barber. The identity of the cultures obtained from apple roots has been established by comparison with cultures grown from ascospores taken from mature stromata on apple roots and locust stumps.

Xylaria sp. from Harrisonburg is distinct from the two preceding species. In culture it resembles *X. cornu-damae* (Schw.) Berk. obtained from germinated ascospores from locust, but its identity with this species is problematical.

CULTURE FEATURES OF THE XYLARIAS

All species of *Xylaria* grew readily on a variety of nutrient media. Gueguen (6), Harder (8), and Freeman (2) have studied the development of *X. hypoxylon* in culture, and Gueguen (7) has also described pure cultures of *X. polymorpha*. Our experience that stromata of *X. hypoxylon* bearing conidia are produced on a variety of culture media is in agreement with their findings. Freeman, however, states that ascospores were produced in cultures of *X. hypoxylon* on steamed blocks of elm wood in six or eight weeks. We have not succeeded in obtaining ascospores in any of our cultures.

Czapec agar¹ and a starch-agar medium have been used in most of our isolation and culture work, the former being preferable for the production of stromata. The colonies of the three species in petri-dish cultures are easily distinguished. Colonies of *Xylaria hypoxylon* (Pl. 16, D) have considerable aerial mycelium with strongly marked, irregularly concentric zones, irregularly lobed margins, and dark pigmentation. Colonies of *X. polymorpha* and *Xylaria* sp. have appressed mycelium with

¹ Cane sugar, 30.0 gm.; magnesium sulphate, 0.5 gm.; potassium chlorid, 0.5 gm.; potassium acid phosphate, 1.0 gm.; sodium nitrate, 2.0 gm.; iron sulphate, trace; agar, 20.0 gm.; water, 1,000 c. c.

indistinct zonation, entire or slightly lobed margins, and produce pigment only with age or not at all. Colonies of *Xylaria* sp. enlarge much more slowly than those of the two other species. The zonation of colonies of *X. hypoxylon* results from the formation of aerial mycelium at the growing margins followed by appressed mycelium. The pigment, which is at first greenish and becomes almost black with age, forms first in the central zone and appears in the other zones successively from the center outwards. The upright stromata begin to form when the colonies are about 2 weeks old and may appear at the center or on the margins of the zones. They vary in numbers from 2 or 3 to 20 or more. The stromata of *X. hypoxylon* are borne singly or in groups and may become 4 cm. or more in length, with a diameter of 2 or 3 mm. (Pl. 17, E). Conidia are produced at the upper extremities in four or five weeks. Cultures of *X. polymorpha* and *Xylaria* sp. have commonly produced only rudimentary stromata. One culture of *X. polymorpha* has, however, developed stromata 5 cm. long and 2 mm. broad. Gueguen's (7) experience that the development of *X. polymorpha* in culture is correlated with its seasonal development in nature is of interest in this connection. A more detailed study of the production of stromata and conidia is reserved for a later publication.

PATHOGENICITY OF THE XYLARIAS

Little is known of the exact mode of life of the Xylarias in nature. It has probably been assumed very generally that they exist as saprophytes; and the appearance of their fructifications on stumps, fence posts, and similar situations is evidence that they are commonly saprophytic. We have found but one reference which bears on the question of parasitism. Harder (8) obtained evidence of the pathogenicity of *X. hypoxylon* when inoculated into small wounds in short pieces of living 1-year-old shoots of two trees (the species were not named). After three months in moist chambers the shoots were still living, as shown by the development of buds and roots, the bark at the point of inoculation was browned to a distance of 3.5 cm., and the wood beneath was brown and dead. The inoculum was recovered.

Our inoculations were made on living apple roots and branches in moist chambers and in the field, also on roots of peach (*Amygdalus persica*), hawthorn (*Crataegus spathulata*), red oak (*Quercus rubra*), white oak (*Q. alba*), and walnut (*Juglans nigra*) in moist chambers.

MOIST-CHAMBER INOCULATIONS

Pieces of roots or branches about 6 inches long and 1 inch in diameter were used. After a preliminary washing and sterilization in alcohol a small notch was cut with a sterile knife and the inoculum of mycelium from the growing margin of a colony introduced. Moisture was main-

tained with a layer of sterilized sand. Roots of apple, peach, hawthorn, and walnut remained alive and in good condition in these moist chambers for more than three months, often sending out shoots several inches long. The oak roots did not keep so well and showed no adventitious budding. One series included inoculations with isolations of *Xylaria hypoxylon* from seven sources and with *Xylaria* sp. Each moist chamber contained one inoculated apple root, one control root wounded but not inoculated, and one inoculated apple limb. All the strains of *X. hypoxylon* proved pathogenic on apple roots and limbs. On roots the progress of infection was seen in the production of surface mycelium on the bark (Pl. 16, F) radiating outward from the point of inoculation accompanied by drops of brown exudate and some splitting of the bark. The mycelium, at first white, later developed pigment and formed a black stromatic crust over the surface of the root, typical of that found on affected apple roots in nature. The surface mycelium and crust developed most vigorously in a saturated atmosphere. After six weeks the pieces were removed and split longitudinally through the wound. The bark and wood were brown and dead to some distance from the wound (Pl. 16, A). The smallest lesion produced extended 1 cm. and the largest 9 cm. proximally and distally from the point of inoculation. The depth of the lesions varied from 3 mm. to 1.5 cm., sometimes extending to the center of the root. Abundant mycelium was found in the vascular bundles, while but little was present in the medullary rays. All the control roots remained healthy. Additional inoculations have confirmed these results. The inoculum has been recovered with ease whenever attempted.

On limbs the mycelium of *Xylaria hypoxylon* produced more conspicuous surface mats than on the roots (Pl. 16, B) and from which the black encrustation developed (Pl. 16, C). Six-inch lengths of limbs were completely covered with the mycelium in less than six weeks; the wood, however, was only penetrated to a depth of a few millimeters. *Xylaria* sp. proved almost as vigorous a parasite as *X. hypoxylon*. In 10 weeks the root infection had spread 2 cm. proximally and distally and 5 mm. inward, while that on the limb had advanced 3 cm. in the bark and had penetrated the wood 2 mm.

Xylaria hypoxylon proved only slightly pathogenic on roots of red oak, white oak, and walnut. A heavy black crust was formed over the wound, but the wood was penetrated but slightly after three months. The hawthorn root was attacked somewhat more vigorously, the wood having been penetrated to a distance of 1 cm. in three months.

Mycelium of *Xylaria polymorpha* produced only slight penetration of the bark of apple roots after three months, and similar results were obtained with peach roots. Ascospores sown on the wound surface of an apple root produced a small amount of mycelium which did not penetrate the sound wood.

Trichoderma koningi, when placed on wounded living apple roots in a moist chamber, grew for a time on the medium transferred, but produced no penetration of the root. *Sphaeropsis malorum*, however, proved pathogenic on apple roots, but the infection progressed more slowly than that produced with *Xylaria hypoxylon*. The lesion had extended 1.5 cm. proximally and distally and to a depth of 5 mm. after four weeks (Pl. 15, E). Recovery in pure culture was obtained.

FIELD INOCULATIONS

Results similar to those obtained in moist chambers have been obtained in inoculations of living apple roots in the field. A number of 1-year-old apple trees were inoculated with *Xylaria hypoxylon* through a small bark wound near the base of the main root and then planted. A few of these examined after four months all showed infection. One bore a lesion 1.5 cm. long which had penetrated to the center of the root. *X. hypoxylon* was recovered from the margin of the lesion. A number of roots of a 20-year-old apple tree were cut across about 5 feet from the trunk, and mycelium of *X. hypoxylon* was inserted beneath the bark at the cut end. The wound was covered with cotton and the soil replaced. Two of these roots examined after seven weeks had been killed 4 cm. from the wound and bore the typical black encrustations on the surface. Another series of roots were inoculated through small bark wounds. After five weeks infection was well established and was advancing into living tissue.

CONCLUSIONS FROM INOCULATION STUDIES

Xylaria hypoxylon has proved to be an active wound parasite on apple roots; *Xylaria* sp. from Harrisonburg is also a wound parasite, and *X. polymorpha* appears to be only slightly or occasionally parasitic. *Sphaeropsis malorum* is the only other fungus obtained from apple roots that has proven parasitic. The isolation of *X. hypoxylon* from roots of affected trees from 9 of the 11 orchards under observation and the experimental evidence of its pathogenicity, accompanied by the production of symptoms in inoculated roots typical of those found in the orchard, indicate that this fungus is the common cause of the black rootrot of the apple in Virginia. Confirmatory evidence must be obtained in the production of typical symptoms and death in growing apple trees from pure-culture inoculations. This is provided for in a series of 500 young apple trees inoculated and planted during the past year (1916) at Blacksburg. Positive results from these inoculations can not be expected for a year or more, since the disease progresses slowly in nature.

CONTROL OF BLACK ROOTROT

No control measures of proved value are known. There is little possibility of effecting the cure of diseased trees. Those showing but

slight foliage symptoms of rootrot are found to have the infection of the root system so well established that the removal of infected parts from the tree and soil seems a practical impossibility. Control measures must therefore be directed toward the prevention of replant infection and further spread of the infection to healthy trees. Experiments with soil disinfection on a small scale offered but little promise. Replants set after soil treatment with some of the common disinfectants died as rapidly as those on untreated soil. Enough work on this line has not been carried on, however, to warrant general conclusions. Tests of the susceptibility of different rootstocks is suggested as a promising line of experiment.

It has been the practice among some orchardists to lime the soil from which trees affected with rootrot were removed before replanting. We have not secured sufficient data to judge of the value of this treatment, but it seems probable from laboratory studies that an alkaline soil is less favorable for the growth of species of *Xylaria* than an acid soil. The radius of colonies of *X. hypoxylon* after three weeks' growth on starch-agar media, which ranged in reaction between -20° and $+10^{\circ}$ Fuller's scale was as follows: $-20^{\circ}=2.6$ cm.; $-10^{\circ}=3.0$ cm.; neutral= 3.0 cm.; $+10^{\circ}=4.5$ cm. Similarly the addition of hydrated lime ($\text{Ca}(\text{OH})_2$) to the medium produced a check in the growth of colonies of *X. hypoxylon*, the radius of the colony on the untreated medium at 17 days being 4.0 cm., while that on the medium receiving hydrated lime was 2.5 cm.

Field observations indicate that infection is commonly distributed through the orchard in cultivation. The establishment of quarantined areas, where the disease is confined to limited blocks of trees, and the withholding of cultivation within these areas, thus preventing the carrying over of infective material to the noninfected areas, should prove of value in some cases. The removal of borers without disinfection of the knife may readily transmit infection from diseased to healthy trees. It further seems quite probable that the infection of healthy trees may result from the intermingling of their roots with those of diseased trees, but infection would probably progress slowly in this manner.

The *Xylarias* are commonly present on stumps of forest trees, and rootrot is consequently most severe on newly cleared land. It seems that the use of cultivated land only for orchard sites may be of considerable practical importance as a preventive measure.

SUMMARY

Black rootrot of the apple is an infectious disease of very considerable economic importance which has become a serious menace in the chief orchard sections of Virginia.

Foliage symptoms of the disease are not markedly different from those produced by injuries due to other agencies, but the black encrustations

on the surface of affected roots and the accompanying dark zonations in the bark and wood are reliable diagnostic features.

Field observations show that rootrot is infectious. The progress of the disease indicates an attack of a comparatively slow working parasite. Two or more years of infection are probably required to produce the death of the tree.

Apple trees planted on newly cleared land are more liable to attack than those on land cleared and cultivated for some time before planting.

Three species of *Xylaria* have been obtained in pure culture from affected apple roots. *X. hypoxylon* obtained from orchards at nine places proved to be an active wound parasite on living apple roots in moist chambers and in the field. Typical black rootrot symptoms were produced and recovery in pure culture was obtained. *Xylaria* sp. (undetermined) from one source also proved pathogenic. *X. polymorpha* from two orchards at one place proved only slightly, if at all, pathogenic. *X. hypoxylon* is probably the chief cause of black rootrot of the apple in Virginia.

Exclusion practices are suggested as control measures of probable value.

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PLATE 15

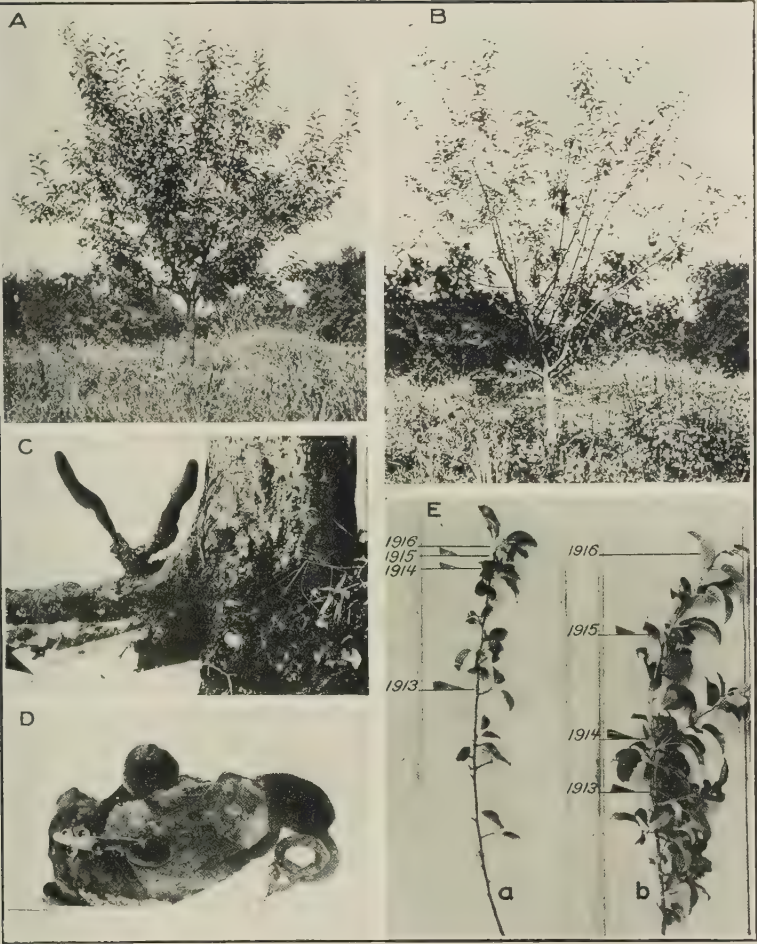
A.—A healthy 6-year-old apple tree contiguous to the affected tree shown in figure B, which appears in the background.

B.—A 6-year-old apple tree showing pronounced symptoms of rootrot in the thinness of the foliage and inclination of the trunk.

C.—*Xylaria polymorpha* fruiting on large lateral root of a 7-year-old apple tree.

D.—*Xylaria hypoxylon* developing stromata on an apple root after three months in a moist chamber.

E.—Terminal growths of trees shown in figures A and B, showing effect of rootrot on annual increase in length: *a*, branch from the tree affected by rootrot; *b*, branch from the healthy tree.



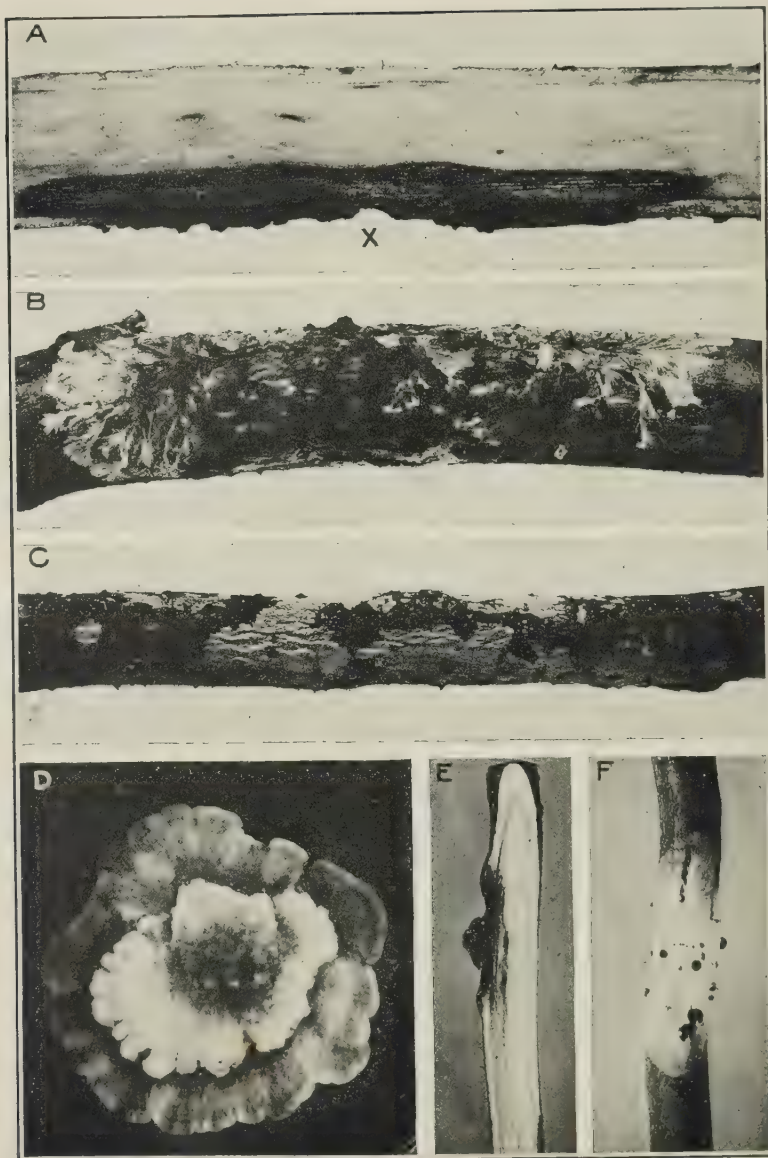


PLATE 16

A.—A longitudinal section of a living apple root inoculated at X with mycelium of *X. hypoxylon* showing discoloration of wood and bark, after six weeks in a moist chamber.

B.—Surface view of a living apple limb inoculated with *X. hypoxylon* after five weeks in moist chamber.

C.—Black encrustation produced on an apple limb inoculated with *X. hypoxylon* in a moist chamber.

D.—An 8-day old colony of *X. hypoxylon* on starch agar showing characteristic zonation and lobed margin.

E.—A longitudinal section of a living apple root inoculated with *Sphaeropsis malorum*, after four weeks in a moist chamber. A black mat of mycelium has formed over point of inoculation.

F.—A surface view of a living apple root inoculated with mycelium of *X. hypoxylon*, after four weeks in moist chamber.

PLATE 17

A.—*Xylaria hypoxylon* fruiting at the base of a dead apple tree.

B.—Stumps of young apple trees which have died from rootrot after having been planted where old trees had died from the same cause. Note black encrustations. Photographed by Dr. H. S. Reed.

C.—Black rootrot lesions on an apple root from Cloverdale, Va. The margin between diseased and sound parts is sharply marked. *X. hypoxylon* was obtained from these lesions.

D.—Longitudinal section of a stroma of *X. polymorpha*, showing perithecia embedded in the periphery.

E.—Conidial stromata of *X. hypoxylon* in petri-dish culture on Czapek agar. The direction of growth is positively phototropic.

F.—Mature stromata of *X. hypoxylon* from stump shown in figure A, producing ascospores after one month in a moist chamber.

